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Maize-*Crotalaria spectabilis* intercropping in organic system and relations with the insect community

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Abstract

The objective of this study was to evaluate the canopy insect community associated with maize intercropped with different arrangements of *Crotalaria spectabilis* and the effects on the damage caused to maize by *Spodoptera frugiperda* and *Helicoverpa zea*. The treatments were intercropping systems of maize with *Crotalaria spectabilis*: CR - *Crotalaria spectabilis* sown on the same rows as maize; CBR - *Crotalaria spectabilis* sown between the rows of maize; CRBR - *Crotalaria spectabilis* sown on the rows and between the rows of maize, and M - maize monocrop. The experimental plot consisted of five rows of maize, five meters long, spaced 0.8 m apart. Assessments were made of the following parameters: insect community present in the maize canopy, leaf damage caused by the fall armyworm (*S. frugiperda*) and the corn earworm (*H. zea*), maize grain yield and shoot dry weight of maize and crotalaria. The CRBR intercropping system was characterized by the presence of predators and parasitoids, especially from the families Forficulidae and Braconidae: 79% and 82%, respectively. The maize monocrop, in turn, was mainly characterized by the presence of chewing and sucking phytophagous insects and predators. There was no influence of plant arrangements on the damage to maize caused by *S. frugiperda* (mean variation between 0.47 and 0.64 of damage) and *H. zea* (ranging between 6.42 and 7.49 of damage), neither on the grain yield of the crop (variation between 4129.57 kg ha^{-1} and 5653.77 kg ha^{-1}). Our results suggest that *C. spectabilis* sown in the rows and between the rows of maize has the potential to optimize conservative biological control, without, however, affecting the grain yield of the cereal.

Keywords: biodiversity; canopy insects; guilds; multivariate analysis; *Zea Mays* L.
Abbreviations: CBR_*Crotalaria spectabilis* sown between the rows of maize; CPI_chewing phytophagous insects; CRBR_*Crotalaria spectabilis* sown in the rows and between the rows of maize; CR_*Crotalaria spectabilis* sown in the same rows as maize; *C. spectabilis_Crotalaria spectabilis; H. zea_Helicoverpa zea*; LDA_linear discriminant analysis; LDC_linear discriminant coefficients; LD_linear discriminant functions; M_maize monocrop; PCoA_principal component analysis; PC_principal component; SDW_shoot dry weight; *S. frugiperda_Spodoptera frugiperda*; SPI_sucking phytophagous insects.

Introduction

One of the greatest challenges to organic maize (*Zea mays* L.) cultivation is to manage the fall armyworm *Spodoptera frugiperda* (J.E. Smith) and the corn earworm *Helicoverpa zea* (Boddie). Because fall armyworms damage plants during their early development, they are among the pests that most compromise the yield and quality of maize grains (Silva et al., 2018). The caterpillars of the genus *Helicoverpa* ssp. cause not only direct damage, by feeding on stigmata-styles and milky grains, but also indirect damage to corn ears, facilitating the action of secondary pests, such as cornsilk flies *Euxesta* spp (Rezende, 1982; Farrar Jr. et al., 2009; Bertolaccini et al., 2018). In addition, they increase the chance of infection by fungi, e.g., *Aspergillus flavus*, which produce aflatoxins, thereby reducing grain quality (Farias et al., 2014). The increase in biodiversity is one of the premises for insect pest management in an organic system (Altieri et al., 2017). Cropping systems that use several plant species in the same area, such as intercropping and integrated systems, generally have better control of insect pest populations, unlike the outcomes from simplified systems, as is the case with monocropping (Finch and Collier, 2012; Madembo et al., 2020). Plant diversification increases species richness and/or abundance of natural enemies of agricultural pests (Resende et al., 2014; Moreira et al., 2016), as it helps form different microclimates that consequently become refuge areas, and provide alternative foods, such as pollen and nectar (Lopes et al., 2011; Gurr et al., 2017; Landaures et al., 2017). Alternatives to increase plant diversity in agricultural systems include the practice of green manure in a crop rotation system, succession cropping or intercropping with agricultural crops (Gaba et al., 2015). Intercropping of maize with crotalaria (*Crotalaria spectabilis* Roth), referred to as the Santa Brígida System, has already been used by farmers in the non-organic (conventional) system of maize cultivation. In this system, crotalaria is cultivated simultaneously with maize, without, however, affecting growth, development andgrain yield (Oliveira et al., 2010). Additionally, the genus *Crotalaria* has already been acknowledged by the scientific community and farmers for increasing the diversity of natural enemies in diversified production systems (Ratnadass et al., 2012; Hinds and Hooks, 2013). However, little is known about the insects of agricultural interest associated with maize-crotalaria intercropping, both in organic and conventional managements. This is partly due to the complexity of the plant-insect interaction, because the location and plant density of each species in the intercropping system can modify the population dynamics of phytophagous insects and/or natural enemies (Ferguson et al., 1984; Stinner, 1988).

Thus, the objective of this research was to evaluate the insect community present in the canopy of maize plants intercropped with different arrangements of *Crotalaria spectabilis,* and the effects of the damage caused to maize by caterpillars of *Spodoptera frugiperda* and *Helicoverpa zea*.

Results

Grain yield and dry weight of maize and crotalaria

The arrangements for *C. spectabilis* intercropped with maize did not interfere ($p>0.05$) with grain yield (Table 2). For shoot dry weight (SDW), there was a difference ($p<0.05$) between plant arrangements (Table 2). The maize monocrop had higher values compared to the CR and CRBR arrangements, but there was no difference compared to CBR. When maize was grown in the absence of crotalaria, SDW production was 24% higher compared to the CRBR treatment, which showed less accumulation (Table 2). However, crotalaria was not influenced by maize in SDW production (Table 2), as there was no difference ($p>0.05$) among the study arrangements.

Relative frequency of taxonomic groups

Table 3 shows the taxonomic composition of the insect community. The family that most frequently stood out in the group of predators, in all maize cultivation systems, was Forficulidae. Among chewing phytophagous insects, the most frequent families in all cropping systems were Crysomelidae and Ulidiidae. The Ulidiidae family showed a higher frequency in the CBR treatment.

Community of insects separated into guilds

The multivariate model, used to describe the community of insects separated into guilds in the different maize-*C. spectabilis* intercropping systems and maize monocrop, accounted for a large part of the biological variability in the area. The first and second linear discriminant functions (LD1 and LD2) showed linear correlations of 64% and 27%, respectively. The contribution of each guild to the separation of the different cropping systems is expressed by Principal Component Analysis (PCoA) in the vector of treatment means. The linear discriminant coefficients (Table 4) show that, in (LD1), the groups were discriminated mainly by parasitoids, owing to their higher coefficient. In the second function (LD2), the parasitoid and chewing phytophagous insect groups were discriminated, as they have higher coefficients. The ratio of (LD1) to (LD2) for the linear discriminant coefficients (LDC) of the insect diversity attributes, relative to the different maize cropping systems, showed that in the first function (LD1), the intercropping systems CBR and CR were similar and were discriminated from the CRBR and M (maize monocrop) systems owing to the greater presence of parasitoids (Figure 1). For the second function (LD2), treatment M was discriminated by chewing phytophagous insects. In turn, the CBR and CR systems are similar, as there was an overlap of confidence ellipses (Figure 1).

Principal component analysis

According to Principal Component Analysis, the first principal component (PC1) was characterized positively by predators and negatively by parasitoids as they had higher coefficients (Table 4). The second principal component (PC2) was mainly negatively characterized by sucking phytophagous insects and predators, and the third component (PC3) was negatively characterized by chewing phytophagous insects and positively characterized by sucking phytophagous insects (Table 4). The ratio between the components (PC1) and (PC2), indicated that the cultivation systems were similar in terms of the guilds (Figure 2A). The CRBR intercropping system was characterized by PC1 and showed high values of predators and parasitoids. The CBR and CR intercropping systems were related to sucking and chewing phytophagous insects and parasitoids while M (maize monocrop) was related to predators.

The ratio of components (PC1) and (PC3) (Figure 2B) indicates that the M system was characterized by sucking and chewing phytophagous insects, and predators. The CBR intercropping was characterized by parasitoids and predators while the CR intercropping, by chewing phytophagous insects and predators. The ratio of components (PC2) and (PC3) (Figure 2C) corroborated the previous results and did not add additional information. In summary, according to Principal Component Analysis, the maize cropping systems are every similar. However, they can be characterized as follows: CRBR is characterized by predators and parasitoids; CBR and CR are characterized by all guilds and, M (maize monocrop) is characterized mainly by chewing and sucking phytophagous insects, and predators.

Damage caused by S. frugiperda and H. zea

When assessing the damage caused to maize by *S. frugiperda* and *H. zea*, there was no significant difference ($p>0.05$) among the intercropping systems (Table 5). The average scores for damage caused by *S. frugiperda* were low, regardless of phenological stage, probably owing to the meteorological conditions in the region (Table 1). In contrast, the average scores for ear damage caused by *H. zea* were high (Table 5), ranging between 6.42 (CBR) and 7.49 (CR).

Discussion

There was a reduction ($p<0.05$) in the SDW of maize in the arrangements in which crotalaria was sown in the planting row of maize. This result can be due to greater competition on the part of crotalaria when it is sown closer to the maize plants; thus, there was an overlap in the area of nutrient and water absorption (Zanine and Santos, 2004). Despite its excellent competition skills, maize is affected by intercropping, which results in reduced growth, especially in the early stages (Gallo et al., 2017). The lowest initial growth rate occurs because the number of dividing cells is small, resulting in a smaller leaf area and, therefore, lesser use of solar radiation (Braz et al., 2005). Despite competition from

Table 1. Rainfall (RF), temperature (T) and relative humidity (RH) during the months of the experiment. Araras, SP, 2017.

Months	---- RF (mm) ----		---------- T (ºC) ---------			--------- RH (%) ---------		
	Total	Mean	Mean	Max	Min	Mean	Max	Min
Jan/17	254.80	8.22	22.65	28.07	19.36	87.20	96.77	53.86
Feb/17*	54.60	1.95	24.59	30.78	19.64	87.86	99.99	64.08
Mar/17*	203.90	6.58	23.06	29.59	18.52	88.84	100.00	63.59
Apr/17	92.30	3.08	21.69	27.63	17.02	89.75	99.98	68.29

*Months of collection. Source: Automatic Weather Station CCA/UFSCar - EMA (2017).

Table 2. Grain yield of maize and shoot dry weight (SDW) of maize and Crotalaria according to the intercropping arrangements. Araras, SP, 2017.

Treatments	Grain yield	SDW-Maize	SDW-Crotalaria
	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}
Maize	4129.57^{ns}	2710.92 a	-
Maize + CR	4934.14	2125.80 b	905.70^{ns}
Maize + CBR	5199.28	2491.08 ab	694.20
Maize + CRBR	5356.77	2077.20 b	803.30
CV (%)	19.04	13.56	32.95

^{ns}Non-significant, according to the F-test at a 5% probability level. CR, crotalaria sown in the maize row; CBR, crotalaria sown between the maize rows; CRBR, crotalaria sown in the row and between the rows of maize. CV (%) = Coefficient of variation.

Table 3. Relative frequency of taxonomic groups (family level) (%) in the maize monocrop (M) and in the different plant arrangements of *Crotalaria spectabilis* in intercropping with maize. Araras, SP, 2017.

	CBR	CR	CRBR	M	Total
	%				
Predators					
Carabidae	0.02	0.03	0.03	0.02	0.03
Dolichopodidae	0.05	0.08	0.05	0.04	0.06
Forficulidae	0.81	0.75	0.79	0.82	0.79
Cleridae	0.00	-	-	-	-
Coccinellidae	0.06	0.07	0.07	0.06	0.07
Hemerobiidae	0.00	0.01	-	0.00	0.00
Mantiidae	0.00	-	-	-	0.00
Reduviidae	0.01	0.02	0.02	0.03	0.02
Micropezidae	0.02	0.02	0.01	0.02	0.01
Syrphidae	0.03	0.02	0.02	0.01	0.02
Parasitoids					
Braconidae	0.80	0.71	0.82	0.76	0.77
Ichneumonidae	0.08	0.13	0.05	0.16	0.11
Pompilidae	0.04	0.06	-	-	0.03
Vespidae	0.08	0.10	0.14	0.08	0.10
Sucking phytophagous insects					
Alydidae	0.04	0.03	0.08	0.07	0.05
Cercopidae	0.04	0.08	0.02	0.03	0.04
Cicadellidae	0.30	0.45	0.39	0.43	0.39
Coreidae	0.31	0.08	0.15	0.19	0.18
Pyrrhocoridae	0.03	0.04	0.04	0.04	0.04
Pentatomidae	0.28	0.33	0.32	0.24	0.29
Chewing phytophagous insects					
Arctiidae	0.05	0.04	0.04	0.02	0.04
Argidae	-	0.01	-	0.01	0.00
Dasytidae	0.10	0.10	0.07	0.05	0.08
Chrysomelidae	0.26	0.30	0.39	0.30	0.31
Tenebrionidae	0.01	0.01	0.01	0.01	0.01
Ommexechidae	0.05	0.10	0.11	0.09	0.09
Noctuidae	0.01	0.01	-	0.04	0.01
Ulidiidae	0.52	0.43	0.37	0.47	0.45

CBR, crotalaria sown between the maize rows; CL, crotalaria sown in the maize rows; CLE, crotalaria sown in the row and between the rows of maize and M, maize monocrop.

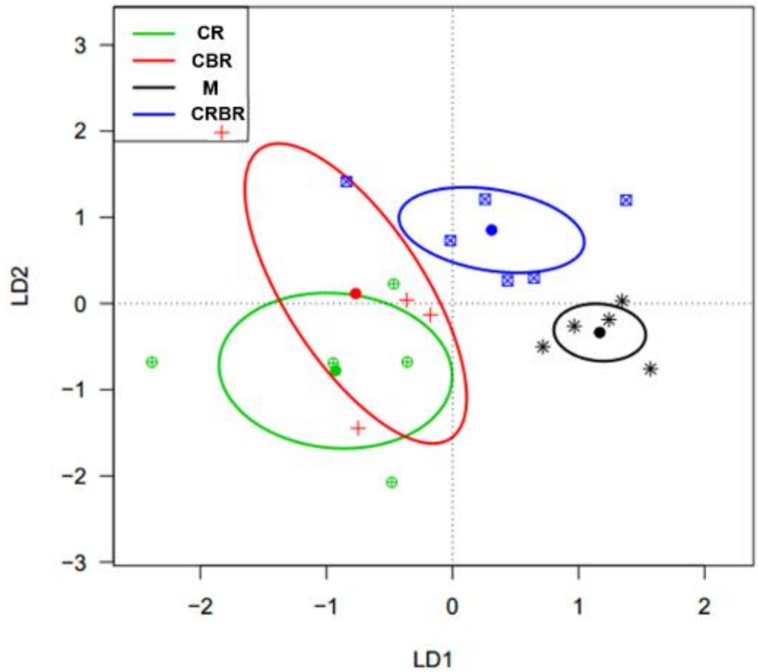

Fig 1. Ratio of the first (LD1) and the second (LD2) discriminating functions, for the different maize-*Crotalaria spectabilis* intercropping systems: (CR) *C. spectabilis* in the maize rows, (CBR) *C. spectabilis* between the maize rows, (CRBR) *C. spectabilis* in the row and between the rows of maize and in the (M) maize monocrop, referring to the linear coefficients of the attributes of the groups of study insects, Araras, SP, 2017.

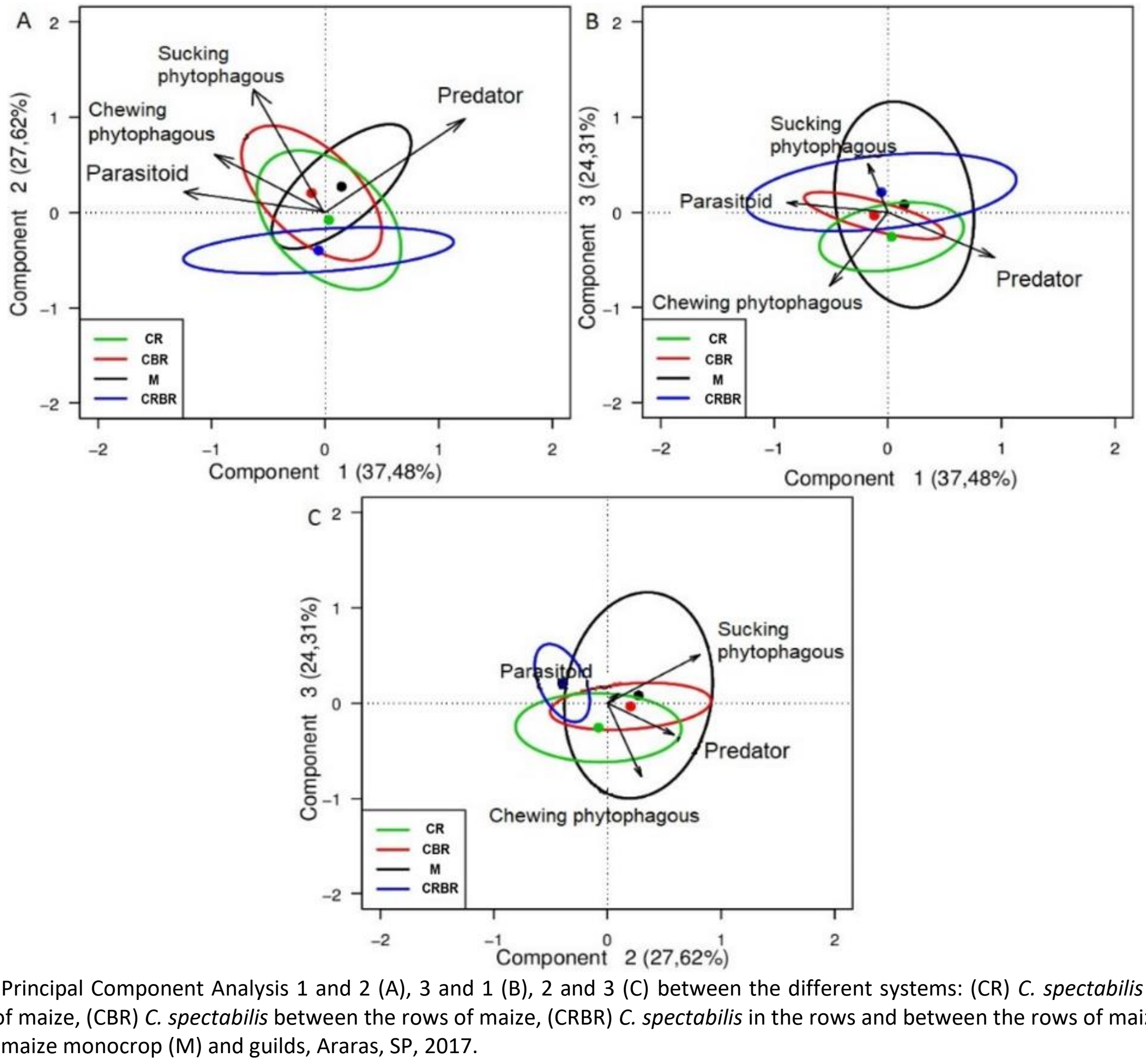

Fig 2. Principal Component Analysis 1 and 2 (A), 3 and 1 (B), 2 and 3 (C) between the different systems: (CR) *C. spectabilis* in the rows of maize, (CBR) *C. spectabilis* between the rows of maize, (CRBR) *C. spectabilis* in the rows and between the rows of maize and in the maize monocrop (M) and guilds, Araras, SP, 2017.

Table 4. List of eigenvectors that characterize the guilds within the principal components (PC1), (PC2) and (PC3), indicating the positive and negative values, and test of comparison of means of the linear discriminant coefficients, for all the attributes of the guilds analyzed in the first (LD1) and the second (LD2) discriminant functions, relative to the different maize-*Crotalaria spectabilis* intercropping systems, and in the maize monocrop, Araras, SP, 2017.

Guilds	LD1	LD2	PC1	PC2	PC3
CPI	-0.02657237	0.189527201	-0.4167717	-0.28314479	-0.7812502
SPI	-0.03259787	-0.008452384	-0.1410502	-0.77522732	0.5152261
Parasitoids	-0.20892112	-0.318947796	-0.7245088	-0.09394324	0.1021081
Predators	-0.06359405	-0.004321382	0.5305593	-0.55680005	-0.3372894

* Independent values greater than the signal (LD1 and LD2). CPI = Chewing phytophagous insects, SPI = Sucking phytophagous insects.

Table 5. Average scores for maize leaf damage caused by *S. frugiperda* in the phenological stages V2 and V4 (2 and 4 expanded leaves) and for the damage in the ear caused by *H. zea* in the phenological stage R4 (Kernel Dough Stage) on the basis of the maize intercropping systems. Araras, SP, 2017.

Systems	*H zea*	*S. frugiperda*	
		V2	V4
CR	7.49^{ns}	0.60^{ns}	0.34^{ns}
CBR	6.42	0.66	0.34
CRBR	6.84	0.64	0.40
M	7.02	0.90	0.38
CV %	7.96	16.24	12.15

CR = *C. spectabilis* in the maize planting rows, CBR = *C. spectabilis* between the maize planting rows, CRBR = *C. spectabilis* in the planting rows and between the planting rows of maize and M = maize monocrop. ^{ns}Non-significant, according to the F-test at a 5% probability level. CV (%) = Coefficient of variation.

crotalaria in the early stages of maize, this influence disappeared over time, since there was no difference (p>0.05) among plant arrangements for grain yield of maize (Table 2). In the taxonomic composition of insects present in the intercropping system (Table 3), one of the most frequent families was Forficulidae. One of the insect species that compose this family is *Doru luteipes*, known as an important predator of major maize pests (Sueldo et al., 2010; Bolzan et al., 2019). The higher occurrence of these insects in the maize monocrop system is probably due to the higher frequency of the Crysomelidae family of insect pests, which is also found in these stages, as they can serve as food for these predators.

In Brazil, Cruz et al. (2011), when evaluating the incidence and the predominant species of *Euxesta* - belonging to the family Ulidiidae - in maize cropping areas, reported an increase in the species *Euxesta eluta* and *E. mazorca* on the ear. According to the authors, the females of these flies oviposit on styles-stigmata and the larvae feed on the developing grains. This behavior is similar to that of *Helicoverpa*. Importantly, at the R1 stage of maize, *C. spectabilis* also had developing pods and grains, which may have led to an increase of the Ulidiidae family in the treatments in which maize was intercropped with the legume between the crop rows.

For multivariate analysis, the results indicate that *C. spectabilis* can provide shelter and food for natural enemies in the agricultural environment, contributing to the control of sucking and chewing phytophagous insects. Plant diversity helps to increase the number of natural enemies, such as predators and parasitoids, providing alternative food resources (Sujii et al., 2010; Snyder, 2019). In addition, some pest insects have smaller populations in more biodiverse systems because the latter make it difficult for the former to locate the host; in addition, changes occur in the microclimate and interfere with insect dispersion (Bastos et al., 2003; Samways et al., 2020). Other factors, e.g., the crotalaria rows acting as a physical barrier for the movement of pests from one row to another, may help reduce insect pest infestation in the intercropping system (Natarajan and Naik, 1992). According to the stability-diversity theory (Sujii et al., 2010), the greater the diversity of organisms in a community, the greater its stability, as there will be food stability for both groups. In the case of the maize monocrop, the greater presence of predators (Figure 2C) can be explained by the presence of sucking and chewing phytophagous insects, which serve as food for them. Populations of other insects are regulated when populations of natural enemies are present in the ecosystem (Dainese et al., 2017). In terms of damage caused by *S. Frugiperda* (Table 5), the intensity of this damage to maize is high when its natural enemies are not present in the cropping area (Harrison et al., 2019). One very important agent in the biological control of insect pests is *Doru luteipes* (Dermaptera: Forficulidae); it is actually the most important in maize crops, as it directly preys on eggs and first-instar caterpillars of *S. frugiperda* (Varella et al., 2015). In the present study, this control may have occurred. Even if there is no difference (p>0.05) for damage among the study arrangements, the number of predators and parasitoids may have increased owing to the presence of crotalaria, as shown in Table 3.

H. zea caterpillars are characterized by locating the host plant by surface texture, odors and contact chemoreceptors. The occurrence of damage to maize intercropped with *C. spectabilis* in the present study is possibly due to the interference caused in the mechanism of *H. zea* for locating the host plant in this type of cultivation. Such a fact may have increased the damage to the ears.

Thus, to predict the effect of increased plant diversity on insects that feed on several simultaneous sources, as in the case of *H. zea*, we must consider the movement of this herbivore in the colonization of the crop and the existence of host preference (Garcia and Altieri, 1992; Montezano and Peil, 2006).

Materials and methods

Location and characterization of the experimental area

The present study was carried out between January and April 2017, in Araras, SP, Brazil, at latitude 22º21'S and longitude 47º23 'W, at 629 m above sea level. The climate is classified as Cwa, mesothermal, with hot and humid summers and dry winters (Köppen, 1948). The climatic data collected during the conduction of the experiment are summarized in Table 1.

The soil of the experimental area was prepared with two harrowing operations (mid-harrow and leveling harrow). The planting furrows were made with a fertilizer-seeder, and maize (hybrid *Pioneer* 30F53) was then sown in the density of six seeds per meter, aiming at a final population of 60,000 plants per hectare, after thinning. *C. spectabilis* was sown simultaneously with maize in the rows and between the rows, according to treatment, in the density of 21 seeds per meter, aiming at a population of seven plants per meter, after thinning. Weed control was carried out using hoes, in the maize development stages V2 (two expanded leaves) and V4 (four expanded leaves), but insects and pathogens were not controlled.

Fertilization was performed by applying the commercial organic compost Visafertil® after sowing maize and crotalaria, on the soil surface, at a dose of 9.2 t ha^{-1} (dry weight), equivalent to 120 kg of nitrogen ha^{-1}.

Experimental design and treatments

The experiment used a randomized block design with five replications. The treatments consisted of three intercropping systems of maize with C. *spectabilis*: CR - C. *spectabilis* sown in the same rows as maize; CBR - C. *spectabilis* sown between the rows of maize; CRBR - C. *spectabilis* sown in the rows and between the rows of maize and a control, M - maize monocrop. The experimental plot consisted of five rows of maize, five meters long, spaced 0.8 m apart.

Assessments of parameters

Assessments were made of the following parameters: insect community present in the maize canopy, leaf damage caused by the fall armyworm (*S. frugiperda*) and the corn earworm (*H. zea*), maize grain yield and shoot dry weight of maize and crotalaria. The insect community present in the corn canopy was assessed at the phenological stages V4 (four expanded leaves), V8 (eight expanded leaves) and R1 (silking). Sampling was carried out by visual search by randomly choosing five points per plot; each point consisted of three meters of maize plants (Cividanes and Yamamoto, 2002), on two consecutive days, in the mornings and evenings, in each phenological stage. After visual inspection, counting and identification of fast-moving insects, manual collections and/or entomological aspiration were carried out. At the R1 stage, an insect net was also used (20 sweeps per plot). The insects were identified at the family level and classified into guilds: predators, parasitoids, sucking phytophagous insects and chewing phytophagous insects, with the help of dichotomous identification keys (Gallo et al., 2002; Triplehorn and Johnson, 2011).

For assessment of leaf damage caused by the fall armyworm (S. *frugiperda*), ten maize plants were sampled per plot in stages V2 and V4, using a rating scale ranging from 0 = no damage to 9 = completely destroyed armyworm (Davis and Williams, 1992). The corn earworm (*H. zea*) was assessed at phenological stage R4 (kernel dough stage) in ten ears, from the three central rows, from each plot. The caterpillars were collected and identified, and a modified version of the centimeter scale of Widstrom (1967) was used for assessment of damage to the ears, with scores ranging between: 0 = no damage; 1 = damage only to the stigmata, without reaching the cob; 2 = cob damage, no more than 1 cm below the ear tip and 3 = damage up to 2 cm below the ear tip, with a unit being added for each additional centimeter.

To determine shoot dry weight of maize, five plants per plot were collected, packed in paper bags and dried in a forced air ventilation oven at ± 65ºC to constant weight, for about 72 hours. The crotalaria plants were cut close to the ground using a 0.50 m quadrat randomly placed in the useable area of the plot (two central rows). The collected material was stored in a paper bag and taken to a forced air ventilation greenhouse at ± 65º C to constant weight. Then, the dry weight of the samples was weighed in a semi-analytical balance. Grain yield was determined after threshing. All grains per plot were weighed on a semi-analytical balance.

Statistical analysis

To assess the community in the canopy, the data underwent Linear Discriminant Analysis (LDA), with characterization of the first and second discriminant functions (LD1 and LD2) related and expressed by the linear discriminant coefficients (LDC). The values of (LDC), in the different linear discriminant functions, underwent the test of comparison of means to identify differences among the treatments through the quality matrix of the adjustment of the discriminant analysis. Then, to characterize the treatments according to the evaluated guilds, the principal components were analyzed in the mean vector of the treatments, following the criterion of Kaiser (1958). Multivariate analyses were performed with the Software R (R Core Team, 2018).

For the other variables, the data underwent analysis of variance, and the means were compared by Tukey's test, at a level of 5% probability, with the aid of the Sisvar software for Windows, version 4 (Ferreira, 2014).

Conclusion

The maize-*C. spectablis* intercropping arrangements affected the insect community present in the crop canopy. The maize-*C. spectablis* intercropping on the rows and between the rows of maize was characterized by the presence of predators and parasitoids, indicating potential use for biological control. The maize monocrop was characterized by guilds of chewing as well as sucking phytophagous insects, and predators, indicating limitations for biological control in this system. There was no effect of the intercropping systems on the damage caused by *S. frugiperda* and H. *zea.*

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